

CYTOLOGICAL STUDIES OF CORNUS

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With plate 53

THERE ARE some forty or more species of *Cornus*, of which some thirty forms are in cultivation at the Arnold Arboretum. This genus has quite a wide distribution all through the northern hemisphere, with one species in the tropical mountains of Africa (Wangerin 1910). According to Berry (1923) over fifty fossil forms have been described, the oldest of these coming from the Upper Cretaceous period; the majority of other forms have been found all through the Tertiary formations.

Out of fifty existing forms described by Wangerin (1910) and Rehder (1927) twenty-four are found in central and eastern Asia, two in western Asia, one in western Asia and Europe, ten in Atlantic North America, six in Pacific North America, three in Central America, one in Alaska and middle western United States, one in Africa and two in the boreal and arctic circumpolar region. All but the last two mentioned are woody while the latter forms are perennial herbs.

Twenty-three species of *Cornus* and one species of *Nyssa* were studied and their chromosome numbers determined. *Cornus canadensis* material was procured from the woods near Pepperell, Mass. This species was studied from aceto-carmin smear preparations of pollen mother cells. The other species were from the Arboretum plants. Five of these were studied both from root-tip section preparations and from aceto-carmin smears. The others were determined from root-tip sections. Representative forms are illustrated in Plate 53. The technique of obtaining root-tips for cytological preparations is described in an earlier publication (Dermen 1931).

A table is given below showing the basis of the taxonomic grouping of the genus *Cornus* based on Rehder's classification (1927), but with slight alterations, and the corresponding chromosome grouping of the species investigated.

TABLE I.

- A. Flowers in cymes or panicles without an involucre.
 - B. Leaves alternate.....(10 pairs of chromosomes)
Cornus alternifolia, *C. controversa*.
 - BB. Leaves opposite.....(11 pairs of chromosomes)
Cornus alba, *C. alba Rosenthalii*, *C. stolonifera*, *C. stolonifera flaviramea*, *C. stolonifera coloradensis*, *C. rugosa*, *C. Amomum*, *C. obliqua*, *C. arnoldiana*, *C. asperifolia*, *C. glabrata*, *C. racemosa*, *C. paucinervis*, *C. coreana*, *C. sanguinea*, *C. Bretschneideri*.

- AA. Flowers in dense umbels with an involucre.
 B. Flowers yellow with a yellowish involucre not exceeding the flowers and deciduous during anthesis.....(9 pairs of chromosomes)
Cornus mas, C. officinalis.
- BB. Flowers greenish yellow with large white or pink bracts.
 C. Woody plants.....(11 pairs of chromosomes)
Cornus florida, C. kousa chinensis.
- CC. Herbaceous plant.....(22 pairs of chromosomes)
Cornus canadensis.

From the above table it is seen that chromosome numbers correspond to the system of taxonomic grouping. This investigation showed four groups of species with basic chromosome numbers 9, 10, 11, 22. In Plate 53 are illustrated meiotic and mitotic figures as representative of cytological groups. Meiotic chromosomes of *C. mas* (fig. 1) are considerably larger than the meiotic chromosomes of *C. florida* (fig. 2) and much larger than the chromosomes of *C. canadensis* (fig. 3). These size differences are also noticeable in the somatic chromosomes (*C. mas*, fig. 4; *C. florida*, fig. 5). Both meiotic and somatic chromosomes of *C. officinalis* correspond in size and structure to the *C. mas* chromosomes. *Cornus kousa chinensis* (fig. 6) was similar to *C. florida* in all respects; both had one pair of chromosomes with minute trabants. There were no trabants observed in any other species. The chromosomes of the *C. alba* group were somewhat smaller than the chromosomes of the *C. mas* and *C. florida* groups. In this, as well as in the *C. florida* group a pair of chromosomes were noticeable that were conspicuously longer than the others, with double constrictions (*C. stolonifera*, fig. 7; *C. stolonifera flaviramea*, fig. 8). Most of the other chromosomes had subterminal constrictions (*C. paucinervis*, fig. 9). *Cornus controversa* (fig. 10) and *C. alternifolia* have 20 chromosomes. In these species there were found four chromosomes that were much longer than the others in the cell. It was difficult to find a cell with chromosomes in a flat position making it possible to illustrate this point; however, with careful observation these differences could be noticed. Winge (1917) has reported for *C. racemosa* (*C. candidissima*) the chromosome count $n = 8-9$ and for *C. glabrata* $n = 11-12$. Both these species have 11 pairs of chromosomes.

Attempts were made to study other genera that are classified either within the family Cornaceae or in Nyssaceae to see in what respects they may have affinities to the genus *Cornus*. *Nyssa sylvatica* was the only species available for a study of chromosome number, size, and structure. This species has 44 chromosomes (fig. 11) quite small in size, but showing at least two pairs of chromosomes noticeably longer than the others. Several efforts were

made to study *Helwingia japonica* and *Davidia involucrata* (the only species of these genera available in the Arboretum) but the chromosomes were found too crowded together, thus making an accurate count difficult. The chromosome number for *Davidia* was estimated $40 \pm$ and for *Helwingia* $80 \pm$. Both had small chromosomes like *Nyssa*. The root-tip cells of *Cornus*, *Nyssa* and *Davidia* were of the same nature; half of them were thick walled showing the presence of a gummy substance. *Helwingia* did not possess these gum cells.

Cornus mas (fig. 4) and *C. officinalis* have 9 pairs of chromosomes. The somatic chromosomes of these forms indicate the presence of 2 pairs with median or submedian constrictions. These 3 pairs are longer than the other chromosomes of the group. When other forms are compared with these, one finds that all the chromosomes are quite short with only one long pair and with a double constriction. From these facts it may be concluded that the two median constricted pairs were segmented, giving rise to the four extra chromosomes of the 11 pair group. The chromosomes of the 10 pair group showed two pairs that were considerably longer than the others. In this case, apparently only one of the nine pairs was segmented to give rise to this additional pair. The 22 pairs of chromosomes of *C. canadensis* undoubtedly are from the duplication of the 11 pairs of some species like *C. florida* or some herbaceous diploid form. To shed some light on the origin of this species with a tetraploid number of chromosomes, the author intends to study *C. suecica*, another herbaceous species, and some other varieties of *C. canadensis*.

From the chromosome counts and structure it is suggested that nine pairs may be taken as the basic number of the genus and that other forms with 10, 11, and 22 pairs are merely alterations of this basic number due to segmentation of some chromosomes and duplication in the case of *C. canadensis*. There is evidence in supporting the hypothesis that fragmentation of chromosomes may give rise to new forms. Stern (1928) gives a case described by Seiler that clearly demonstrates this point. There were found two races of butterflies, *Phragmatobia fuliginosa*, one with 28 and the other with 29 pairs of chromosomes. Seiler finds that the long chromosomes of the 28 pair race are four units long, while in the 29 pair race the long chromosomes are three units long. When these races are crossed he finds that long chromosomes pair with the fragmented chromosomes. Anderson (1931) has made a comparative study of the chromosomes of the genera *Allium* and *Nothoscordum*. The genus *Nothoscordum* is considered closely

related to the genus *Allium*. In *A. stellatum* he finds seven pairs of chromosomes, while the characteristic number for the genus is eight pairs. In *N. bivalve* are found nine pairs. In the nine chromosomes of the microspore, seven were with median or sub-median constrictions and two with terminal constrictions. In the words of the author, "These latter are conspicuously marked by large, deep-staining insertion points. The chromosomes, like those of *Allium*, are large and ribbon-like. The attachment constrictions in *Allium* are usually median or sub-median (or at most sub-terminal). It seems quite possible that *Nothoscordum* may have been derived from an eight-chromosomed parental stock by the division of one of the large median-constricted chromosomes. This is further borne out by the fact that the combined length of the two chromosomes with terminal constrictions is only a very little greater than that of the longest chromosome with a median constriction." Thus it may be assumed that when a long chromosome is fragmented from the point of so-called "spindle fiber attachment point," then the derived chromosomes build up anew their own spindle fiber attachment constrictions.

It may be safe to assume that a species with a small number of chromosomes is the most primitive of its genus. Taking the cytological findings based on number, size and structure of chromosomes, it may be said that a type like *C. mas* is the most primitive of the genus and others are derivatives of this, both in respect to cytological characteristics and morphology of inflorescence.

Unfortunately, due chiefly to difficulties of cultivation, the Arboretum does not have some of the species like *C. Volkensii*, *C. cilicia*, *C. Nuttallii*, etc. that could have helped to make this study more complete. These species mentioned are of special interest because of their geographical distribution and because, in some cases, of their close resemblance to other forms, like *C. Nuttallii* to *C. florida*, the former growing in Pacific United States and the latter in Atlantic United States.

The present distribution of these species suggests that *Cornus* was an ancient genus dating back into the Lower Cretaceous period, before, according to geographical formations discussed by Fernald (1931), the Arctic Ocean had connection with the Gulf of Mexico, when there were supposed to be land connections between North America and Europe, Africa was connected with Europe and Transcaucasia was the land bridge between Europe and Asia. The facts that *C. mas* has the least number of chromosomes and other points indicated above, and that there are many varieties of *C. mas* growing all through that region, and that Transcaucasia

is in the middle point of the early geographical formation of the earth, indicate that forms with higher number of chromosomes may have been derived from *C. mas* and these forms spread to the left through Europe and America and to the right through Asia and down to the present African tropical mountains. Two species, *C. alternifolia* and *C. controversa* (alternate leafed), with two additional chromosomes are very similar. The former has moved to the west occupying Atlantic North America and the latter moved to the present tropical regions of the East Indies, China, Korea and Japan. Other forms with four additional chromosomes must have originated from *C. mas*, changing morphologically in some respects but retaining the opposite position of leaves. Other species with 11 pairs of chromosomes are most likely derivative forms of a species like *C. florida* or may very well be direct descendants of *C. mas*.

It was stated above that *Nyssa sylvatica* had 44 chromosomes and that there were observed two pairs of chromosomes considerably longer than others. If this observation is correct, then this genus may be considered a derivative form of a *Cornus* species with 11 pairs of chromosomes.

In connection with the chromosome study of *Cornus*, pollen grains of 25 species were measured and the percentage of pollen grain abnormality was determined.

TABLE II.

Name of Species	Per Cent of Pollen Grain Abnormality	Measurement in μ
<i>C. alba Rosenthalii</i>	15	215
<i>C. alba Kesselringii</i>	50	260
<i>C. stolonifera</i>	2	215
<i>C. stolonifera flaviramea</i>	2	215
<i>C. stolonifera coloradensis</i>	5	260
<i>C. rugosa</i>	30	205
<i>C. Slavinii</i> *	15	215
<i>C. Amomum</i>	30	280
<i>C. dubia</i> *	65	245
<i>C. obliqua</i>	4	250
<i>C. arnoldiana</i> *	80	190
<i>C. asperifolia</i>	25	200
<i>C. Dunbarii</i> *	75	215
<i>C. glabrata</i>	2	215
<i>C. racemosa</i>	50	180
<i>C. coreana</i>	10	215
<i>C. Bretschneideri</i>	2	205
<i>C. florida</i>	10	170
<i>C. florida rubra</i>	95	160
<i>C. kousa</i>	70	135
<i>C. kousa chinensis</i>	3	125
<i>C. mas</i>	3	110
<i>C. mas flava</i>	3	110
<i>C. officinalis</i>	2	110
<i>C. canadensis</i>	10	110

In the above table are given the species that were studied and recorded. Species with asterisks are hybrids. These are given in Rehder's Manual of Cultivated Trees and Shrubs, 1927. The measurements in microns are 180–280 for *C. alba* group, 125–170 for *C. florida* group, 110 for *C. mas* group, and 110 for *C. canadensis*. This record shows most strikingly that chromosome number and size do not control the size of pollen grains, while on the other hand, each group has its characteristic measurement. It was also found that *C. mas*, *C. florida*, *C. kousa*, *C. officinalis*, and their varieties develop their pollen grains in the fall, while others develop theirs in the spring. In this respect there was found an affinity between the *C. mas* and *C. florida* groups. *Cornus canadensis*, while related to *C. florida*, develops its pollen grains in the spring. This difference may be due to its being an herbaceous form.

From all the above facts it is suggested that *C. mas* or a similar species is the most primitive type; that the *C. alba* group may be considered as a derivative group from the *C. florida* type or directly from the *C. mas* type; and that the *C. florida* and *C. alternifolia* groups are parallel derivatives from the *C. mas* type. *Cornus canadensis* should be considered a derivative from the *C. florida* type. At present nothing can be said concerning *Davidia*, only that the root-tip cells were similar to *Cornus* and *Nyssa*, and that the chromosome number is estimated to be $40 \pm$.

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DESCRIPTION OF PLATE 53

- Fig. 1. *C. mas*. Second metaphase plate showing at one pole 9 chromosomes.
- Fig. 2. *C. florida*. First metaphase plate with $n = 11$ chromosomes.
- Fig. 3. *C. canadensis*. First metaphase plate with $n = 22$ chromosomes.
- Fig. 4. *C. mas*. Metaphase plate from root-tip section with $2n = 18$ chromosomes.
- Fig. 5. *C. florida*. Metaphase plate from root-tip section with $2n = 22$ chromosomes.
- Fig. 6. *C. kousa chinensis*. Metaphase plate from root-tip section with $2n = 22$ chromosomes.
- Fig. 7. *C. stolonifera*. Metaphase plate from root-tip section with $2n = 22$ chromosomes.
- Fig. 8. *C. stolonifera flaviramea*. Metaphase plate from root-tip section with $2n = 22$ chromosomes.
- Fig. 9. *C. paucinervis*. Metaphase plate from root-tip section with $2n = 22$ chromosomes.
- Fig. 10. *C. controversa*. Metaphase plate from root-tip section with $2n = 20$ chromosomes.
- Fig. 11. *Nyssa sylvatica*. Metaphase plate from root-tip section with $2n = 44$ chromosomes.